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"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

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The overall goals of this project as specified in NIH-NINDS-94-10, were as follows: to design, fabricate and test both discrete activated iridium microelectrodes and University of Michigan silicon microelectrodes suitable for chronic stimulation of cortical neurons; to study the effects of cerebral cortical microstimulation on surrounding neural, vascular, and on extracellular fluid, using both physiologic and histologic evaluation; and to attempt to minimize mechanical trauma due to electrode insertion by evaluations of tip configuration, type of insertion, speed of insertion, and the use of single vs multiple electrode arrays.

Passive Implants

During the first year-and-one-half, we undertook a systematic program to minimize the trauma due to the insertion of both iridium and University of Michigan silicon microelectrodes. Using only passive (non-pulsed) electrodes (QPR's 1-6), the following parameters were evaluated: (1) Four different tip configurations with iridium microelectrodes 50 μm in diameter with 1 μm faceted, 3, 6, and 12 μm conical tips; (2) Rapid electrode insertion using a stereotaxic frame-mounted axial introducer at speeds up to 108 cm/sec; (3) Manual introduction using padded forceps; (4) The use of a proteolytic enzyme cocktail to soften the pia in order to facilitate insertion of the electrodes and reduce dimpling of the cortical surface; (5) The use of single and multiple iridium arrays; (6) Comparison of acute (4-24 hrs.) and chronic (2-3 weeks) evaluations; and finally, (7) Evaluation of the use of fibrin glue or saline-soaked Gelfoam "blankets" and artificial dura vs. dural reapproximation over the dural incision.

These studies resulted in a number of significant findings which have enabled a substantial reduction in the trauma of microelectrode insertion into the cerebral cortex. First, the acute studies (4 or 24 hour implantation) with iridium microelectrodes, the 1, 3 or 6 μm tips almost invariably showed microhemorrhages associated with the tracks of the microelectrode. In general, these microhemorrhages occurred irrespective of the tip configuration or the rate of insertion. Although implementation of the use of fibrin glue to overlay the operative sites in place of suturing the dura significantly reduced the incidences of microhemorrhages even in acute experiments. In one study, implants of 6 iridium and 43 University of Michigan electrodes

resulted in a marked reduction of the number and severity of interstitial hemorrhages (5 hemorrhages associated with 49 tracks).

In later studies we evaluated iridium electrodes with blunter tips (12 μm in diameter), which elicited much fewer microhemorrhages and neuronal damage than did the sharper 1-6 μm diameter electrodes after acute implants (QPR #5).

With respect to sharp vs. blunt iridium electrodes, the sharper tip (faceted electrodes with 3 and 6 μm conical tips) were accompanied by more microhemorrhages, and slight to moderate numbers of shrunken, hyperchromic neurons compared to completely normal-appearing neurons at all tracks of the blunt-tipped microelectrodes (QPR #5). The use of a proteolytic enzyme cocktail to soften the pial surface to lessen penetration force and reduce dimpling of the pia did not reduce the incidence of microhemorrhages. The enzymes did succeed in partly digesting the walls of the leptomingeal blood vessels resulting in bleeding within the meninges and occasionally this extended into the cortical molecular layer. Microhemorrhages at the tips of the electrodes were significantly reduced by omission of dural suturing (which was replaced, first by covering the implant site with fibrin glue and in later experiments, by a square of artificial dura (silicone elastomer)). This approach reduced microhemorrhages by apparently minimizing movement of the electrode array. Sealing of the dura is rapid, and no untoward effects of CSF leakage or damage to the underlying pia and molecular layer of the cortex have been observed following this procedure.

Histologic evaluation of the chronic implants (2-3 weeks after implantation of the electrode), revealed the electrode sites in a regenerative state, yet large cavitations presumably resulting from rupture of larger vessels in the cortical microcirculation, followed by scavenging of extravasated erythrocytes, were still very easily detectable. For these reasons, acute implantations (4-24 hours) were abandoned in lieu of the more informative chronic evaluations (QPR's 6-8 and 10).

Blunt-tipped 12 μm electrodes greatly reduced mechanical injury, although some displacement and flattening of neurons were consistently noted at the tip sites. To address this problem we reduced the thickness of the iridium shafts from 50 to 35 μm in diameter and assembled the electrode in arrays of 7 (QPR's 8 and 10). In these two series of animals, there was

a marked decrease in the number of microhemorrhages and interstitial cavitations at 3 weeks after implantation of the arrays. Minimal microhemorrhages have been confirmed in subsequent experiments in which the electrodes with blunt tips were pulsed.

The factors responsible for these improved results are attributed to a combination of: (1) omitting suturing of the dura and the substituting of fibrin glue, or artificial dura, (2) use of a more blunt (12 μm) electrode tip, (3) reduction of the iridium shaft size from 50 to 35 μm, (4) the use of an array of 7 electrodes (6 in a circle with a longer electrode in the center) appears to increase the overall positional stability of individual electrodes, and finally, (5) the use of a vacuum operated electrode inserter rather than a stereotaxic frame mounted axial introducer for microelectrode insertion.

Pulsed Electrodes (University of Michigan Silicon Substrate).

In addition to passive implants with silicon electrodes, we have begun to implant arrays having 4 tines and 16 stimulating sites (three cats QPR #'s 7 & 8). Silicon, multisite electrodes were pulsed continuously for 7 hours with charge densities of 2,000 $\mu C/cm^2$ (8nC/phase, $5A/cm^2$). In two of these animals (QPR #7) there was an accumulation of lymphocytes around the pulsed sites. Two of the pulsed electrode sites failed (became open circuited) during the 7-hour stimulation after remaining functional in vivo for 33 days. These results suggested that a stimulus charge per phase of 8nC was excessive for the silicon substrate electrodes (QPR-7) arrays. In the third animal, the same stimulation protocol induced no electrically-induced injury nor accumulation of lymphocytes.

The primary undesirable consequence of the prolonged, continuous stimulation with silicon electrodes was a marked depression in the electrical excitability of neurons near the micro-electrode tips. We have not yet examined interleaved stimulants using these electrodes. It was also encouraging that all 3 arrays with multielectrode sites showed minimal mechanically induced damage, confirming the observations with the unpulsed electrodes described above.

Pulsed Iridium 7 Electrode Arrays

Activated iridium microelectrodes in arrays of seven have been pulsed for 7 hours with a range of parameters and then evaluated histologically (QPR #10). In most cases gliosis was sparse at the electrode tips following implants of 45 to 140 days. However in a few electrodes in 3 animals, the gliosis appeared as packed aggregates of astrocytes suggesting some type of contamination on the electrode shafts.

By far, the predominant histologic feature of this series was an accumulation of lymphocytes around the tips of pulsed electrodes (but none, or only a very few, lymphocytes at the tips of unpulsed controls). Reducing the stimulus frequency from 400 to 50 Hz did not reduce the size of the lymphocytic aggregates nor did interleaved pulsing significantly reduce the lymphocytic aggregations. The largest lymphocytic aggregations were invariably accompanied by decreased neuronal excitability as assessed by recording of the compound action potentials from the corticospinal tract. In a recent study in one animal 5 electrodes were pulsed in the interleaved mode for 7 hours at 25 Hz, 16 μA and 2.4 nC/ph) and 2 were unpulsed. There were no lymphocytes at the unpulsed electrodes and a diminished lymphocytic accumulation (compared to 8nC in previous studies) at the 5 pulsed electrode sites. Thus there is a positive correlation between the magnitude of charge/ph and the lymphocytic aggregation. More studies are needed. It is unclear whether the lymphocytic aggregation is due to a true electrotaxic phenomenon or whether the lymphocytes are attracted to cytokines released by glia or other cells, or to a substance produced electrochemically at the electrode-electrolyte interface.

Summary of Observations of Tissue Injury During Prolonged Cortical Microstimulation

We have identified several types of histologic and physiologic effects of prolonged microstimulation which might be detrimental to the long-term functionality of sensory or motor prostheses or at least may degrade their performance. We have designated these as Level 1, 2 and 3 effects. Level 1 effects are those in which there are reversible changes in the functioning of the neurons near the microelectrodes (e.g., stimulation-induced depression of neuronal excitability), but no histologically-detectable tissue injury. The stimulation-induced depression of neuronal excitability (SIDNE) may persist for several days, although the effect is ultimately reversible if it is not accompanied by obvious histologic changes.

Level 2 effects are histologically-detectable changes associated with the stimulation, and which are not obviously detrimental, but which need further investigation to determine their significance. A conspicuous level 2 effect is the infiltration of a small number of lymphocytes into the tissue around pulsed (and sometimes unpulsed) microelectrodes in the cerebral cortex. As long as the lymphocytes do not aggregate densely around the tip, there is no obvious evidence of tissue injury.

Level 3 effects are histologic changes which are certainly detrimental, and are accompanied by marked increase in the threshold of electrically-induced responses from the implanted electrodes. Following prolonged stimulation of the brain with epicortical macroelectrodes, neurons subjacent to the electrode disks may become shrunken and hyperchromic to the histologic stains. In the cerebral cortex, we have observed dense aggregates of lymphocytes around pulsed electrodes, accompanied by displacement and disruption of occasional neurons and neuropil. In the cochlear nucleus, we have observed microvacuolations of the myelinated axons close to microelectrodes that have been pulsed at high amplitude and at a high rate.

Level 1, 2 and 3 effects may be superimposed, and collectively, these phenomena can be designated as "stimulation-induced neural damage and dysfunction" (SINDD).

In our pending contract period, we will determine the electrode configurations, stimulus parameters and stimulus regimens that can activate the required neuronal population while minimizing SINDD.

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